



IS GAIN-OF-FUNCTION RESEARCH UNLOCKING THE SECRETS OF HIDDEN DANGEROUS VIRUSES?

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ABSTRACT

Gain-of-function experiments may help medical researchers to test scientific theories, develop new technologies and find treatments for infectious diseases. When the original SARS-CoV outbreak occurred in 2003, scientists developed a method to study the virus in the laboratory, by growing the virus in mice. This endeavour showed a model for researching the virus and testing potential vaccines and treatments. Inadequate and insufficient research on viruses will authorize unaware of future pandemics. But too little consciousness of security threats will multiply the hazards that an experiment virus may escape a lab through an accident and cause an outbreak of its own. A virus generated in the lab by genetically modified its gene products, altering an organism, and if accidentally released, could result in millions of deaths.

Gain-of-Function research is followed in scientific studies to cautiously understand the possibility of the emergence of infectious viruses in nature. Gain-of-Function experiments include engineering in the lab genetic alterations that simulate an increase in virulence. The organism modified this way is tested on laboratory animals like ferrets, mice, and guinea pigs. These tests provide insight that is utilized to develop vaccines, test out new antivirals, understand the host-immune response, and comprehend the disease-causing ability of the virus. In this way, we can remain a step ahead of viral evolution by developing suitable medical countermeasures. Gain-of-function research is the serial passaging of microorganisms to increase transmissibility, virulence, immunogenicity, and host tropism.

KEY WORDS: Gain-of-Function, mice, and guinea pigs, immunogenicity, Dual-use research of concern (DURC)

Introduction

GOFR is a subset of "dual-use research"—i.e., research that can be used for both beneficial and malevolent purposes (1,2)

Dual-use research of concern (DURC) is one of the most hotly debated science policy issues during the 21st century, with controversy surrounding a series of published experiments with potential implications for biological weapons-making.(3)

Gain-of-function research (GoF research or GoFR) is medical research that genetically alters an organism in a way that may enhance the biological functions of gene products. (4)

Introducing a mutation that would allow influenza B to infect rabbits in a controlled laboratory situation would be considered a gain-of-function experiment, as the virus did not previously have that function (5)

That type of experiment could then help reveal which parts of the virus's genome correspond to the species that it can infect, enabling the creation of antiviral medicines which block this function (6)

In virology, gain-of-function research is usually employed with the intention of better understanding current and future pandemics. In vaccine development, gain-of-function research is conducted in the hope of gaining a head start on a virus and being able to develop a vaccine or therapeutic before it emerges (7) The term "gain of function" is to refer to a pathogen to replicate more quickly or cause more harm in humans or other closely-related mammals (8)

The term first gained a wide public audience in 2012, after two groups revealed that they had tweaked an avian influenza virus, using genetic engineering and directed evolution, until it could be transmitted between ferrets (9)

The experiments did make the virus amenable to research, however, and the team shared both it and the engineered mice with others. It led to plenty of new findings. (10)

Responses with SARS-CoV-2, suggesting that if interferon is provided as a treatment, it should be early in the course of the disease (11)

Researchers also used the mouse-adapted MERS-CoV to test new vaccines and treatments. The Iowa team's collaborators tested a vaccine that is a hybrid of

parainfluenza virus with the MERS-CoV version of the spike. The vaccine wasn't very effective when injected, but it did protect DPP4-expressing mice from MERS-CoV quite well when provided through the nose (12)

Although MERS outbreaks haven't led to sustained transmission, this information has proved valuable in the COVID-19 pandemic: a vaccine with the same design, but against the SARS-CoV-2 spike, works in mice and ferrets and is now undergoing early clinical trials (13)

Parainfluenza virus type 5 (PIV5) is a negative-stranded RNA virus in the family Paramyxoviridae that has been evaluated as a vaccine vector for influenza, respiratory syncytial virus (RSV), rabies, and a variety of other pathogens (14,15).

In animal models, PIV5 is safe and is not associated with any disease, with the exception of kennel cough in dogs (16).

Intranasally administered kennel cough vaccines containing live PIV5 have been used for more than four decades with an excellent safety record. Dogs immunized with kennel cough vaccines can shed PIV5 for up to 5 days, and it has been safe to humans in close contact with immunized animals (17,18).

In January 2020, the National Science Advisory Board for Biosecurity convened an expert panel to revisit the rules for gain-of-function research and provide more clarity in how such experiments are approved, and when they should be disclosed to the public (19,20)

The origins of GOFR

The term "gain of function" refers to "research which could enable a pandemic-potential pathogen to replicate more quickly or cause more harm in humans or other closely-related mammals."

What does gain of function mean?

Any organism can acquire a new ability or property, or "gain" a "function."

Gain of function can also be useful for environmental reasons, such as modifying *E. coli* so that it can convert plastic waste into a valuable commodity.

In the current debate around SARS-CoV-2, the virus that causes COVID-19, gain of function has a much narrower meaning related to a virus becoming easier to move between humans, or becoming more lethal in humans. It is important to remember, though, that the term "gain of function" by itself covers much more

than this type of research.

Any organism can acquire a new ability or property, or "gain" a "function." Some lab examples include creating more salt- and drought-resistant plants or modifying disease vectors to produce mosquitoes that are resistant to transmitting dengue fever. Gain of function can also be useful for environmental reasons, such as modifying *E. coli* so that it can convert plastic waste into a valuable commodity.

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Why would researchers do gain-of-function work on potentially dangerous pathogens?

Gain-of-function experiments may help researchers test scientific theories, develop new technologies and find treatments for infectious diseases. For example, in 2003, when the original SARS-CoV outbreak occurred, researchers developed a method to study the virus in the laboratory.

One of the experiments was to grow the virus in mice so they could study it. This work led to a model for researching the virus and testing potential vaccines and treatments.

Gain-of-function research that focuses on potential pandemic pathogens has been supported on the premise that it will help researchers better understand the evolving pathogenic landscape, be better prepared for a pandemic response and develop treatments and countermeasures.

What are some examples of gain-of-function research, and how risky is it? Potential Benefits

Some potential outcomes of gain-of-function research may include the creation of organisms that are more transmissible or more virulent than the original organism or those that evade current detection methods and available treatments.

Other examples include engineering organisms that can evade current detection methods and available treatments, or grow in another part of an organism, such as the ability to cross the blood-brain barrier.

Risk and Challenges

Gain of function experiments make viruses more infectious to humans in the laboratory settings. This research look like gamble that civilization can't afford to risk. There is no such thing as zero risk in conducting experiments. So the question is whether certain gain-of-function research can be performed at an acceptable level of safety and security by utilizing risk-mitigation measures.

A robust biosafety and biosecurity system, along with appropriate institutional response, helps to ensure that these incidents are inconsequential. The challenge is to make sure that any research conducted – gain-of-function or otherwise – doesn't pose unreasonable risks to researchers, the public and the environment.

Function research and global pandemic

In virology, gain-of-function research is usually employed with the intention of better understanding current and future pandemics.

In vaccine development, gain-of-function research is conducted in the hope of gaining a head start on a virus and being able to develop a vaccine or therapeutic before it emerges.

The term "gain of function" is sometimes applied more narrowly to refer to "research which could enable a pandemic-potential pathogen to replicate more quickly or cause more harm in humans or other closely-related mammals."

Some forms of gain-of-function research (specifically work which involves certain select agent pathogens) carry inherent biosafety and biosecurity risks, and are thus also referred to as dual use research of concern (DURC).

During the COVID-19 pandemic a number of highly speculative theories spread about the origin of the SARS-CoV-2 virus and links to gain-of-function research. In January 2021, University of Saskatchewan virologist Angela Rasmussen wrote that one version of the information invoked previous gain-of-function work on coronaviruses to promulgate the idea that the virus was of laboratory origin

Gain-of-function (GOF) research involves experimentation that aims or is expected to (and/or, perhaps, actually does) increase the transmissibility and/or virulence of pathogens. Such research, when conducted by responsible scientists, usually aims to improve understanding of disease causing agents, their interaction with human hosts, and/or their potential to cause pandemics. (21).

The last—and only—foreign scientist in the Wuhan lab speaks out
An expert in bat-borne viruses, Danielle Anderson is the only foreign scientist to

have undertaken research at the Wuhan Institute of Virology's BSL-4 lab, the first in mainland China equipped to handle the planet's deadliest pathogens. Danielle Anderson was working in what has become the world's most notorious laboratory just weeks before the first known cases of Covid-19 emerged in central China. Yet, the Australian virologist still wonders what she missed.(22)

Covid-19 was a 'man-made' virus, claims scientist who worked at the Wuhan lab

With merely any biosafety provisions, China's gain-of-function experiments — which are conducted to genetically alter organisms in order to enhance their biological functions — led to a leak at the Wuhan lab. A US-based scientist, who worked at a research lab based in China's Wuhan revealed Monday that Covid-19 was a "man-made virus" that had leaked from the facility, according to a report in The New York Post.(23)

The Lab-Leak Theory: Inside the Fight to Uncover COVID-19's Origins

As cities worldwide were shutting down to halt the spread of COVID-19, Demaneuf, 52, began reading up on the origins of SARS-CoV-2, the virus that causes the disease. The Huanan wholesale market, in the city of Wuhan, is a complex of markets selling seafood, meat, fruit, and vegetables. A handful of vendors sold live wild animals—a possible source of the virus.(24)

NeCoV coronavirus found in bats may pose threat to humans in future, scientists caution

NeCoV is closely related to the Middle East respiratory syndrome, a viral disease first identified in Saudi Arabia in 2012. A single molecular change in the lab enabled a coronavirus called Neocov to "efficiently infect" human cells using the same pathway that the SARS-CoV-2 uses to infect human cells, researchers from Wuhan University, Wuhan, China said in a report that is yet to be peer-reviewed.

Neocov has so far only been seen in bats and no instances have been reported in people, but being closely related to the Middle Eastern Respiratory Syndrome (MERS) coronaviruses —traditionally more lethal but less transmissible than Sarscov2 --the study has raised concern that this too may lethally proliferate in people . Experts however say that such fears are unwarranted.(25)

Application of Gof Research

Current medical countermeasures are often insufficient largely. Viruses develop resistance mechanisms that lead to "escape mutants," that is, drug-resistant strains and the current medical countermeasures are often insufficient. There is, a need to develop new antiviral drugs like, immunotherapy, based on neutralizing monoclonal antibodies. The GoF studies, boostup viral yield and immunogenicity, are required for vaccine development. Molecular methods help with the characterization of antigenic variants, elucidate the biological basis for adverse outcomes associated with vaccine candidates, and determine the basis for attenuation and stability of vaccine candidates

Alternative Research Methods with Potentially Less Risk

- Molecular dynamical modelling of influenza proteins and interactions with inhibitors and receptor
- In vitro studies of specific properties required for human adaptation, using single proteins
- In vitro studies of genetic interactions between loci in one or several viral proteins using replication-incompetent viruses – epistatic interactions
- Sequence database comparisons of genetic properties of human and avian adapted viruses
- Comparisons of human seasonal isolates and zoonotic isolates from infected humans and avian isolates

'Not convinced' Covid-19 developed naturally, says Dr Fauci

--America's top expert on infectious diseases, Dr Anthony Fauci said he is "not convinced" the novel coronavirus developed naturally and has called for an open investigation into the origins of Covid-19 virus.

When Dr Fauci was asked at an event whether he was still confident that the coronavirus developed naturally he said: "I am not convinced about that, I think we should continue to investigate what went on in China until we continue to find out to the best of our ability what happened."

"Certainly, the people who investigated it say it likely was the emergence from an animal reservoir that then infected individuals, but it could have been something else, and we need to find that out. So, you know, that's the reason why I said I'm perfectly in favour of any investigation that looks into the origin of the virus," Dr Fauci was quoted by Fox News.(26)

COVID cracked out from Wuhan Institute of Virology (WIV), laboratory

In what appears to be an astonishing revelation, a US-based scientist, who worked at a controversial research lab in China's Wuhan, has said that COVID-19 was a "man-made virus" that leaked from the facility.

COVID was leaked from Wuhan Institute of Virology (WIV), a state-run and funded research facility, two years ago, the New York Post reported, quoting US-based researcher Andrew Huff's statement in the British newspaper The Sun.

In his latest book, "The Truth About Wuhan," epidemiologist Huff claims that the pandemic was caused by the US government's funding of coronaviruses in China. Excerpts of Huff's book have been out in the UK-based tabloid The Sun. According to the New York Post report, Huff is the former vice president of the EcoHealth Alliance, a non-profit organisation based in New York that studies infectious diseases (27)

Origins of SARS-CoV-2: Why the lab-leak idea is being considered again.

Since the beginning of the COVID-19 pandemic, there have been various hypotheses to explain the origin of SARS-CoV-2.

So far, none of these hypotheses have shown the origins of the virus. One of the first scenarios put forward — that the Wuhan seafood market allowed for a rapid dispersion of the virus — seems less certain now. After a year of intensive research, the virus has still not been identified in any animals. Animal-human transmission, however, remains one of the strongest hypotheses explaining the appearance of the new coronavirus: the coronavirus was transmitted from bats to humans through an intermediate host. It would not be the first time this happened. In the case of MERS-CoV (Middle East Respiratory Syndrome), camels likely served as the intermediate host. For SARS-CoV-2, pangolins, scaly anteaters sold illegally in the Wuhan market, may be the host, although this hypothesis requires more convincing evidence.(28)

Conclusion

In virology, gain-of-function research is usually employed with the intention of better understanding current and future pandemics. Gain-of-Function research is followed in scientific studies to cautiously understand the possibility of the emergence of infectious viruses in nature. Gain-of-Function experiments include engineering in the lab genetic alterations that simulate an increase in virulence. The organism modified this way is tested on laboratory animals like ferrets, mice, and guinea pigs. These tests provide insight that is utilized to develop vaccines, test out new antivirals, understand the host-immune response, and comprehend the disease-causing ability of the virus. In this way, we can remain a step ahead of viral evolution by developing suitable medical countermeasures. Gain-of-function research is the serial passaging of microorganisms to increase transmissibility, virulence, immunogenicity, and host tropism. In vaccine development, gain-of-function research is conducted in the hope of gaining a head start on a virus and being able to develop a vaccine or therapeutic before it emerges.

REFERENCES

- Miller S, Selgelid MJ. Ethical and philosophical consideration of the dual-use dilemma in the biological sciences. Dordrecht, NE: Springer; 2008.
- National Research Council (NRC) and Institute of Medicine (IOM) Potential risks and benefits of gain-of-function research: Summary of a workshop. Washington, DC: National Academies Press; 2015
- Jackson RJ, Ramsay AJ, Christensen CD, Beaton S, Hall DF, Ramshaw IA. Expression of mouse interleukin-4 by a recombinant ectromelia virus overcomes genetic resistance to mousepox. *Journal of Virology*. 2001;75:1205–1210
- Osterhaus, A. D.; Rimmelzwaan, G. F.; Martina, B. E.; Bestebroer, T. M.; Fouchier, R. A. (2000-05-12). "Influenza B virus in seals". *Science*. 288 (5468): 1051–1053. Bibcode:2000Sci...288.1051R Retrieved 25 May 2021.
- Gain-of-Function Research: Background and Alternatives. National Academies Press (US). 2015-04-13. Retrieved 25 May 2021
- Imperiale, Michael J.; Howard, Don; Casadevall, Arturo (28 August 2018). The Silver Lining in Gain-of-Function Experiments with Pathogens of Pandemic Potential. *Influenza Virus. Methods in Molecular Biology*. Vol. 1836. pp. 575–587.
- Selgelid, Michael J. (2016-07-06). "Gain-of-Function Research: Ethical Analysis". *Science and Engineering Ethics*. 22 (4): 923–964.
- "Recommended Policy Guidance for Departmental Development of Review Mechanisms for Potential Pandemic Pathogen Care and Oversight (P3CO)" (PDF). Department of Health and Human Services. Office of the Assistant Secretary for Preparedness and Response. 9 January 2017.
- Sander Herfst , Eefje J A Schrauwen, Martin Linster, Salin Chutinimitkul, Airborne transmission of influenza A/H5N1 virus between ferrets, *Science*, 2012 Jun 22;336(6088):1534-41.
- Jeong Seok Lee , Eui-Cheol Shin, The type I interferon response in COVID-19: implications for treatment, *Nat Rev Immunol*. 2020 Oct;20(10):585-586.
- Jeong Seok Lee , Eui-Cheol Shin, The type I interferon response in COVID-19: implications for treatment, *Nat Rev Immunol*. 2020 Oct;20(10):585-586. doi: 10.1038/s41577-020-00429-3
- Kun Li , Zhus Li , Christine Wohlford-Lenane , David K Meyerholz , Rudragouda Channappanavar , Dong An , Stanley Perlman , Paul B McCray Jr Biao He Single-Dose, Intranasal Immunization with Recombinant Parainfluenza Virus 5 Expressing Middle East Respiratory Syndrome Coronavirus (MERS-CoV) Spike Protein Protects Mice from Fatal MERS-CoV Infection, 2020 Apr;7;11(2):e00554-20.
- Dong An; Kun Li; Dawne K. Rowe; Maria Cristina Huertas Diaz; Emily F. Griffin; Ashley C. Beavis; Scott K. Johnson; Ian Padykula; Cheryl A. Jones; Kelsey Briggs et al. Protection of K18-hACE2 mice and ferrets against SARS-CoV-2 challenge by a single-dose mucosal immunization with a parainfluenza virus 5-based COVID-19 vaccine, *Science Advances*, 2021-07-02
- S. I. Phan, Z. Chen, P. Xu, Z. Li, X. Gao, S. L. Foster, M. N. Teng, R. A. Tripp, K. Sakamoto, B. He, A respiratory syncytial virus (RSV) vaccine based on parainfluenza virus 5 (PIV5). *Vaccine* 32, 3050–3057 (2014).
- S. I. Phan, J. R. Zengel, H. Wei, Z. Li, D. Wang, B. He, Parainfluenza virus 5 expressing wild-type or prefusion respiratory syncytial virus (RSV) fusion protein protects mice and cotton rats from RSV challenge. *J. Virol.* 91, e00560-17 (2017).
- A. J. Mooney, Z. Li, J. D. Gabbard, B. He, S. M. Tompkins, Recombinant parainfluenza virus 5 vaccine encoding the influenza virus hemagglutinin protects against H5N1 highly pathogenic avian influenza virus infection following intranasal or intramuscular vaccination of BALB/c mice. *J. Virol.* 87, 363–371 (2013)
- E. J. Kontor, R. J. Wegryzn, R. A. Goodnow, Canine infectious tracheobronchitis: Effects of an intranasal live canine parainfluenza-Bordetella bronchiseptica vaccine on viral shedding and clinical tracheobronchitis (kennel cough). *Am. J. Vet. Res.* 42, 1694–1698 (1981).
- Z. Chen, P. Xu, G. W. Salyards, S. B. Harvey, B. Rada, Z. F. Fu, B. He, Evaluating a parainfluenza virus 5-based vaccine in a host with pre-existing immunity against parainfluenza virus 5. *PLOS ONE* 7, e50144 (2012).
- D. Wang, S. Phan, D. J. Di Stefano, M. P. Citron, C. L. Callahan, L. Indrawati, S. A. Dubey, G. J. Heidecker, D. Govindarajan, X. Liang, B. He, A. S. Espeseth, A single-dose recombinant parainfluenza virus 5-vectored vaccine expressing respiratory syncytial virus (RSV) F or G protein protected cotton rats and african green monkeys from RSV challenge. *J. Virol.* 91, e00066-17 (2017).
- J. Zheng, L.-Y. R. Wong, K. Li, A. K. Verma, M. E. Ortiz, C. Wohlford-Lenane, M. R. Leidinger, C. M. Knudson, D. K. Meyerholz, P. B. McCray Jr., S. Perlman, COVID-19 treatments and pathogenesis including anosmia in K18-hACE2 mice. *Nature* 589, 603 (2019)
- michael J. Selgelid, Gain-of-Function Research: Ethical Analysis, *Sci Eng Ethics*. 2016; 22(4): 923–964. 2016 Aug 8.
- Bloomberg, The last—and only—foreign scientist in the Wuhan lab speaks out June 28, 2021 12:29 IST
- Express Web Desk, Covid-19 was a 'man-made' virus, claims scientist who worked at the Wuhan lab, *The Indian Express*, December 6, 2022 14:30 IST
- KATHERINE EBAN, <https://www.vanityfair.com/news/2021/06/the-lab-leak-theory-inside-the-fight-to-uncover-covid-19s-origins>
- Jacob Koshy, January 28, 2022 05:17 pm | Updated January 29, 2022 12:04 pm IST - Beijing, <https://www.thehindu.com/sci-tech/health/neocov-coronavirus-found-in-bats-may-pose-threat-to-humans-in-future-scientists-caution/article38339161.ece>
- <https://www.deccanchronicle.com/lifestyle/health-and-wellbeing/051222/covid-was-man-made-virus-says-scientist-who-worked-at-wuhan-lab.html>
- 1 IST, <https://www.deccanchronicle.com/lifestyle/health-and-wellbeing/051222/covid-was-man-made-virus-says-scientist-who-worked-at-wuhan-lab.html>
- <https://www.deccanchronicle.com/science/science/070621/origins-of-sars-cov-2-why-the-lab-leak-idea-is-being-considered-again.html>